Nutraceuticals & Food
Vol. 8, p. 119~123 (2003)

Nutraceuticals and Food

The Effects of Carnitine and/or Gamma-Aminobutyric Acid (GABA) Supplementation on the Recovery of Chronic Ethanol Administered Rats

Ju-Ryoun Soh, Tokuo T. Yamamoto* and Youn-Soo Cha†

Department of Food Science and Human Nutrition, and Institute for Molecular Biology and Genetics, Chonbuk National University, Chonju 561-756, Korea
**Tohoku University Gene Research Center, Tohoku University, Sendai 981-8555, Japan*

Abstract

To investigate the effects of the supplementation of carnitine and/or γ-aminobutric acid (GABA), Sprague-Dawley male rats were orally treated with either an AIN-76 diet (control), a control diet plus ethanol (CE, 4 g ethanol/kg bw), CE plus L-carnitine (CEC, 0.5 g/kg bw), CE plus GABA (CEG, 0.5 g/kg bw), or CE plus L-carnitine plus GABA (CECG, 0.25 g/kg bw each) for 6 weeks. Serum triglyceride levels were increased in the CE group and were decreased significantly in the CEC, CEG and CECG groups. HDL-cholesterol was increased and LDL-cholesterol was decreased in the CEG and CECG groups compared with the CE group. Serum GOT and GPT levels increased by the chronic ethanol administration were decreased in the CEC group. In addition, we have evaluated the mRNA levels of carnitine palmitoyltransferase-I in those groups. Supplementation of carnitine/GABA had some recovery effects on the liver CPT-I mRNA levels which decreased by chronic ethanol administration. These results may suggest that supplementations of either L-carnitine or GABA are effective on the recovery of chronic ethanol-related symptoms, but no combined effects were shown.

Key words: carnitine, GABA, ethanol, lipids, CPT-I

INTRODUCTION

There are millions of people throughout the world who responsibly enjoy consuming alcoholic beverages served as food, medicines, and euphoriants. However, alcoholic beverages served as food, medicines, and dependence and other alcohol-related disabilities are some of the most challenging social, economical and public health problems facing our modern-day society (1). It was reported that chronic and/or acute consumption of alcohol is known to cause a wide variety of body abnormalities arising from changes in the metabolism that alter the rewarding effects of alcohol such as hyperlipidemia, fatty liver, euphoria or relaxation (2,3).

Carnitine (β-hydroxy-γ-trimethylamino butyric acid) is an essential cofactor for the transfer of long-chain fatty acids across mitochondrial membranes for β-oxidation (4). Ethanol administration both in humans and laboratory animals results in hyperlipidemia, fatty liver, and ultimately the most severe stage of alcoholic liver disease. Carnitine supplementation lowered ethanol-induced increases of various lipid fractions in rat's liver in a dose related manner (5-9).

γ-Aminobutyric acid (GABA) is a ubiquitous non-protein amino acid that is produced primarily from an α-decarboxylation of L-glutamic acid (Glu) catalyzed by the enzyme glutamate decarboxylase (GAD). It is well known that GABA functions in animals as a major inhibitory neurotransmitter (10,11). GABA is involved in the regulation of cardiovascular functions, such as blood pressure and heart rate, and plays a role in the sensation of pain and anxiety. Many neurological disorders, such as seizures, Parkinson's disease, stiff-man syndrome, and schizophrenia have been shown to be related to alterations of the GABA and GAD levels in the brain. In alcoholics, remarkably low plasma GABA levels and a low expression of GABA receptor in the brain have been measured (12-15).

It was demonstrated that the uptake of GABA by the high-affinity system was competitively inhibited by carnitine, while the low-affinity system was inhibited in a mixed way (16-18). Carnitine up-take in rat brain slices is inhibited by GABA as a result of competition at a common carrier site.

Therefore, we investigated the supplementation of carnitine or GABA and their mixed effects on ethanol metabolism in rats.

†Corresponding author. E-mail: cha8@moak.chonbuk.ac.kr
Phone: +82-63-270-3822. Fax: +82-63-270-3854

MATERIALS AND METHODS

Materials

GABA, Carnitine, DL-methionine, fiber, and choline bitartrate, the total RNA isolation kit were purchased from the Sigma Chemical Co. (St. Louis, USA). AIN-76 vitamin and mineral mix were purchased from Harlan Teklad (Madison, USA). Casein was purchased from Cottee (Gordon, Australia). Psoralen-biotin labeling kit, nylon membrane, and the detection kit were purchased from Ambion (Austin, USA).

Animal and diets

Male Sprague-Dawley rats, aged 4 weeks, were purchased from Daehan Biolink Inc. (Eumsung, Korea). They were fed a normal chow (Jeil-jedaing, Suwon, Korea) for adaptation during a week, then randomly divided into five groups of 6 each; control group, ethanol supplement group, ethanol and carnitine supplement group, ethanol and GABA supplement group, ethanol, carnitine and GABA supplement group. Each group was fed normal AIN-76 purified diet that are shown in Table 1. They were housed individually in stainless steel cages and kept in state of most suitable, temperature (23±1°C), humidity (53±2%), with maintained a 12 hr/12 hr light-dark cycle. The experimental diet and water were provided *ad libitum*. The rats were orally administered carnitine and/or GABA and ethanol once a day for 6 weeks in the supplement groups as shown in Table 2, and given distilled water of the same volume as the control group. The weight of the rats was measured once in a week.

Table 1. Composition of experimental diets[1)]

Ingredient	Percent (%)
Casein	20.0
Sucrose	50.0
Starch	15.0
Corn oil	5.0
Cellulose	5.0
Mineral mixture	3.5
Vitamin mixture	1.0
Choline bitartrate	0.2
DL-methionine	0.3
Total	100.0

[1)]All components are in units of g/100 g diet.

Table 2. Experimental design and sample treatments

Samples	Groups[1)]				
	C	CE	CEC	CEG	CECG
Ethanol (g/kg)	-	4	4	4	4
L-carnitine (g/kg)	-	-	0.5	-	0.25
GABA (g/kg)	-	-	-	0.5	0.25

[1)]C, control diet; CE, C plus ethanol; CEC, CE plus carnitine; CEG, CE plus GABA; CECG, CE plus carnitine plus GABA.

Before all rats were sacrificed, the diet was removed from the cages for 12 hr. Blood was collected by orbital venipuncture and left in ice water for 1 hr. Then blood samples were centrifuged at 1,100×g for 15 min at 4°C, and the serum were stored at -20°C until assayed. The livers were collected, measured and weighed after remove remaining blood, immediately frozen in liquid nitrogen, and stored -80°C until analyzed.

Analysis of lipids and enzyme activities

Serum total cholesterol levels were measured with commercial kits (Asan Pharm. Co., Seoul, Korea) based on the cholesterol oxidase method (19). The HDL-cholesterol fraction was prepared by the dextran sulfate-Mg^{++} method (20) and the HDL-cholesterol level was analyzed enzymatically using commercial kits (Asan Pharm. Co., Seoul, Korea). LDL-cholesterol concentrations were determined by the Friedwald method (21). Serum triglyceride concentrations were measured by the lipase-glycerol phosphate method (22) using commercial kits (Asan Pharm. Co., Seoul, Korea). Serum glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) levels were determined using commercial kits (Asan Pharm. Co., Seoul, Korea). Liver lipids were extracted from liver tissues according to the method of Folch et al. (23) and triglyceride concentrations were measured by the lipase-glycerol phosphate method as described above.

CPT-1 mRNA levels

Hepatic total RNA was isolated by a commercial total RNA isolation kit (Sigma, St. Louis, USA) using the guanidine thiocyanate/silica-based system. The total RNA was quantified by measuring absorption at 260 nm. CPT-I mRNA abundance was measured by Northern blot analysis (24). Total RNA were loaded on 1.2% agarose-formaldehyde gel, electrophoresed and transferred to a nylon membrane (Ambion, Austin, USA). The CPT-I DNA fragment obtained from the CPT-I cDNA, and cDNAs was labeled with biotin using the non-isotopic Psoralen-biotin labeling kit (Ambion, Austin, USA). Hybridization of the probe to the membrane-bound mRNA was performed at 42°C for 22 hrs. The membrane was washed to remove the non-specifically bound probe and incubated in the blocking, conjugation, blocking and CDP-star solution of the BrightStart™ BioDetect™ kit (Ambion, Austin, USA). The membrane was then exposed to X-ray film (Fuji, Tokyo, Japan) for 90 min, at room temperature.

Statistical analysis

Results were expressed as the mean±SD. In this study, differences between means used Statistical Analysis System version 8 (SAS Institute, Cary, NC, USA). The significance of the differences within the five groups were

determined by Duncan's multiple range test and the accepted level of significance was $p<0.05$.

RESULTS AND DISCUSSION

Food intake and body weight gain

Body weight at sacrifice was decreased significantly in the group administered with both the control diet and ethanol compared with the control diet group. However, the significant body weight reduction due to the chronic ethanol administration was not observed in the groups supplemented with carnitine/GABA (Fig. 1). Previously, it was shown that over consumption of ethanol leads to a decrease of body weight (1,25). This is because ethanol lacks essential nutrients, other than calories, which results in primary malnutrition by displacing other nutrients in the diet (26). Secondary malnutrition may be the result from either maldigestion, or malabsorption of nutritions caused by gastrointestinal complications (26,27). This malnutritions can induce deficiency of virtually all of the nutrients (28).

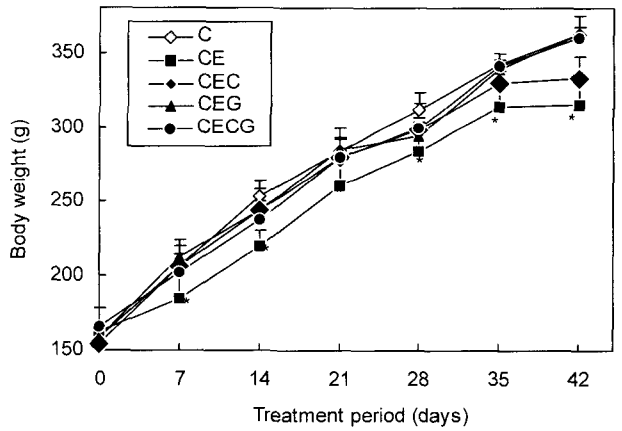

Fig. 1. Body weights of animals during the experimental period. The error bars standard deviation of 6 rats per group.
*Indicates body weight of CE group significant differences from the body weight of the other groups by Duncan's multiple range test ($p>0.05$). C, control diet; CE, C plus ethanol; CEC, CE plus carnitine; CEG, CE plus GABA; CECG, CE plus carnitine plus GABA.

Serum lipid levels

It was observed that carnitine supplementation lowered ethanol-induced increases of various lipid fractions (6). They found that the supplementation of carnitine in a liquid alcohol diet (36% calories as ethanol) significantly lowered total lipids, free and esterified cholesterol, triglycerides, and phospholipids in the liver. In our study, the supplementaion of carnitine/GABA recovered increasing serum triglyceride concentrations due to chronic ethanol administration. Serum HDL-cholesterol concentration in only the GABA supplementation group was higher and LDL-cholesterol concentration was lower than those of the other groups (Table 3). Generally, the measurement of the LDL-cholesterol level in the blood is considered in determining the malfunction of the lipoprotein metabolism, because the LDL-cholesterol level has shown a direct proportional correlation to both coronary heart disease and atherosclerosis (29,30). A mode of action of carnitine is to retard ethanol clearance and hence its metabolism (6-8). Thus, oral administration of carnitine offers protection to rats against ethanol-induced lipid abnormalities in our study. Since GABA showed an inhibiting effect on the peptic output in anaesthetized rats (31), the decrease in serum levels might be due to a retarded absorption and metabolism of ethanol by supplemented GABA.

Serum enzyme activities

It has been shown that ethanol, as well as malnutrition that was induced by ethanol, contributes to liver disease, resulting in a high level of hepatic enzymes in serum (1,28). The supplementation of carnitine/GABA decreased blood GOT and GPT levels that were increased due to the chronic ethanol administration. The effects were stronger in the only carnitine supplementation compared with GABA or carnitine and GABA mixed supplementation groups (Table 3). Several studies have shown that the supplementation of carnitine (0.5% diet) decreased GOT and

Table 3. Concentration of serum lipids and enzymes in rats

Groups	Groups[1)]				
	C	CE	CEC	CEG	CECG
Triglyceride (mg/dL)	81.33±15.0[2)b3)]	151.20±61.9[a]	55.66±15.9[b]	68.70±18.3[b]	77.60±12.5[b]
Total cholesterol (mg/dL)	82.40±13.9	95.60±9.9	78.60±6.8	82.30±13.0	87.00±6.0
HDL-cholesterol (mg/dL)	42.80±5.3[b]	40.20±4.4[b]	38.80±2.7[b]	49.00±6.0[a]	43.10±4.4[ab]
LDL-cholesterol (mg/dL)	33.40±2.0[ab]	38.10±5.1[a]	33.40±4.9[ab]	26.20±11.0[b]	34.90±2.4[ab]
GOT (I.U)	221.5±41.9[ab]	281.5±64.0[a]	210.2±29.8[b]	240.8±29.3[ab]	251.7±51.7[ab]
GPT (I.U)	52.2±10.2[b]	72.4±11.2[a]	55.7±10.2[b]	61.5±8.5[ab]	55.6±2.8[b]

[1)]C, control diet; CE, C plus ethanol; CEC, CE plus carnitine; CEG, CE plus GABA; CECG, CE plus carnitine plus GABA.
[2)]The values represent the mean with standard deviation of 6 rats per group.
[3)]Values with different superscripts are significantly different among the groups ($p<0.05$).

γ -GTP levels increased by ethanol administration (6-8). Also, diet supplemented with plants, or the administration of plant extracts, have an effect on the reduction of serum enzyme activities that were increased by ethanol (25,32) or CCl_4 (33,34). Oh et al. (35) raised the possibility that GABA in plants could have a nutraceutical role in the recovery of chronic alcohol-related diseases. Resently, we found that a diet supplemented with cabbage roots containing a higher level of GABA than the control cabbage decreased plasma γ - GTP level that was increased by the ethanol administration (36). However, chemically pure GABA did not show significant effect on decreasing either serum GOT and GPT levels in this study. Further studies to elucidate the various concentrations of GABA may provide further insights into the approaches for the nutraceutical application of GABA.

Liver CPT-I mRNA level

CPT-I is the rate-limiting enzyme for fatty acid oxidation and is the first step specific to fatty acid oxidation (37). Liver CPT-I activity in chronic ethanol administered rats decreased significantly compared to normal control rats, which indicates that chronic ethanol administered rats in our study have retarded fatty acid oxidation.

Supplementation of carnitine/GABA had some recovery effects on liver CPT-I mRNA levels which decreased by chronic ethanol administration (Fig. 2). The capacity for hepatic fatty acid oxidation was enhanced by fasting, fat feeding, induced diabetes, or the treatment of rats with peroxisomal/mitochondrial proliferating agents. Also, the mRNA and activity levels of CPT-I were increased by modulation of diets (38,39). In a different study, the changes of CPT-I mRNA abundance, produced by hyperthyroidism and hypothyroism, paralleled the changes in CPT-I activity

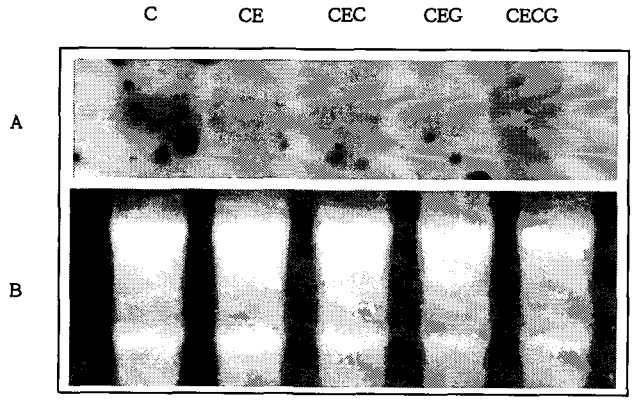

Fig. 2. Hepatic CPT-I mRNA levels. Total RNA was separated by 1.2% agarose gel electrophoresis and transferred to a Nylon membrane. Hybridization and visualization were conducted as described in Materials and Methods. A, Northern blot of mRNA; B, ethidium bromide stain of RNAs; C, control diet; CE, C plus ethanol; CEC, CE plus carnitine; CEG, CE plus GABA; CECG, CE plus carnitine plus GABA.

in the rat liver. This suggests that the CPT-I is regulated at the transcription level by thyroid hormones (24). Transcriptional of the rat liver CPT-I gene was also elevated by both high fat diets and exercise, suggesting that control of the CPT-I gene expression is a key feature in the regulation of fatty acid oxidation during exercise (40).

Overall, this data suggest that carnitie/GABA have beneficial effects on the alcohol induced blood lipids and enzyme activities and the liver CPT-I level. Carnitine and GABA act independently rather than cumulatively on the recovery of alcoholic rats.

ACKNOWLEDGEMENT

This paper was supported in part by research funds of Chonbuk National University.

REFERENCES

1. Lieber CS. 1994. Alcohol and the liver. *Gastro* 106: 1085-1180.
2. Varga K, Gantenberg NS, Kunos G. 1994. Endogenous γ -aminobutyric acid (GABA) mediates ethanol inhibition of vagally mediated reflex bradycardia elicited from aortic baroreceptors. *J Pharmacol Exp Ther* 268: 1057-1062.
3. Morrow AL. 1997. Alcohol's "channels" to the brain. *Center Line* 8: 1-3.
4. Bieber LL. 1988. Carnitine. *Ann Rev Biochem* 57: 261-283.
5. McGarry JD, Foster DW. 1980. Regulation of hepatic fatty acid oxidation and ketone body production. *Ann Rev Biochem* 49: 395-420.
6. Rhew TH, Sachan DS. 1986. Dose-dependent lipotropic effect of carnitine in chronic alcoholic rats. *J Nutr* 116: 2263-2269.
7. Sachan DS, Cha YS. 1994. Acetylcarnitine inhibits alcohol dehydrogenase. *Biochem Biophys Res Comm* 203: 1496-1501.
8. Cha YS, Sachan DS. 1995. Acetylcarnitine-mediated inhibition of ethanol oxidation in hepatocytes. *Alcohol* 12: 289-294.
9. McCaman RE, McCaman MW, Stafford ML. 1996. Carnitine acetyltransferase in nervous tissue. *J Biol Chem* 241: 930-934.
10. Kravitz EA. 1967. *The Neurisctences.* Quarton GC, Melnechuk T, Schmidt FO, eds. Rockefeller University Press, New York. p 433-444.
11. Roberts E. 1975. The basic neurosctences. In *The Nervous System.* Tower DB, ed. Raven Press, New York. Vol I, p 541-552.
12. McGeer PL, McGeer EG. 1976. *GABA in Nervous system Function.* Roberts E, Chase TN, Tower DB, eds. Raven Press, New York. p 487-495.
13. Bao J, Cheung WY, Wu JY. 1995. Brain L-glutamate decarboxylase: Inhibition by phosphorylation and activation by dephosphorylation. *J Biol Chem* 270: 6464-6467.
14. Criswell HE, Simson PE, Duncan GE, McCown TJ, Herbert JS, Morrow AL, Breese GR. 1993. Molecular basis for regionally specific action of ethanol on γ -aminobutyric $acid_A$ receptors: Generalization to other ligand-gated ion channels. *J Pharmacol Exp Ther* 267: 522-537.

15. Tsai GE, Ragan P, Cchang R, Chen S, Linnoila VM, Coyle JT. 1998. Increased glutamatergic neurotransmission and oxidative stress after alcohol withdrawal. *Am J Psychiatry* 155: 726-732.
16. Huth PJ, Schmidt MJ, Hall PV, Fariello RG, Shug AL. 1981. The uptake of carnitine by slices of rat cerebral cortex. *J Neurochem* 36: 715-723.
17. Fariello RG, Shug AL. 1981. Competitive inhibition by 3-amino propanesulfonic acid and γ -aminobutyric acid of carnitine transport in rat brain slices. *Biochem Pharm* 30: 1012-1013.
18. Wawrzenczyk A, Nalecz KA, Nalecz MJ. 1995. Effect of γ -aminobutric acid on the carnitine metabolism in neural cells. *Biochem Biophys Res Commun* 213: 383-388.
19. Allain CC, Poon LS, Chan CS, Richman W, Fu PC. 1974. Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470-475.
20. Warnick JB, Benderson J, Albers JJ. 1978. HDL precipitation by dextran sulfate-$MgCl_2$ method. *Clin Chem* 28: 1379-1383.
21. Friedwald WT, Levy RL, Fredrickso DS. 1972. Estimation of the concentration of low density lipoprotein cholesterol without use of the preparation ultracentrifuge. *Clin Chem* 18: 499-502.
22. McGowan MW, Artiss JD, Strandbergh DR, Zak B. 1983. A peroxidase-couple method for the colorimetric determination of serum triglycerides. *Clin Chem* 29: 538-542.
23. Folch J, Lees M, Sloane-Stanley GHS. 1983. Simple method for the isolation of total lipids from animal tissues. *J Biol Chem* 22: 497-509.
24. Mynatt RL, Park EA, Thorngate PE, Das HD, Cook GA. 1994. Changes in carnitine palmitoyltransferase-I mRNA abundance produced by hyperthyroidism and hypothyroidism parallel changes in activity. *Biochem Biophys Res Commun* 201: 932-937.
25. Oh SH, Cha YS, Choi DS. 1999. Effects of *Angekica gigas* Makai diet on lipid metabolism, alcohol metabolism and liver function of rats administered with chronic ethanol. *J Korean Soc Agric Chem Biotechnol* 42: 29-33.
26. Scheig R. 1970. Effects of ethanol on the liver. *Am J Clin Nutr* 23: 467-473.
27. Mezey E. 1980. Alcoholics liver disease: roles of alcohol and malnutrition. *Am J Clin Nutr* 33: 2709-2718.
28. Lieber CS. 1991. Hepatic, metabolic and toxic effects of ethanol. *Alcohol Clin Exp Res* 15: 573-592.
29. Goldstein JL, Brown MS. 1983. Lipoprotein receptors: Genetic defense against atherosclerosis. *Clin Res* 30: 417-423.
30. Steinberg D, Witztum JL. 1990. Lipoproteins and atherogenesis. *J Am A* 264: 3047-3052.
31. Goel RK, Abbas WR, Maiti RN, Bhattacharya SK. 1996. Effect of continuous infusion of GABA and baclofen on gastric secretion in anaesthetized rats. *Indian J Exp Biol* 34: 978-981.
32. Kim KS, Lee MY. 1996. Effects of *Artemisua selengensis* methanol extract on ethanol-induced hepatotoxicity in rat liver. *J Korean Soc Food Sci Nutr* 25: 581-587.
33. Lee SI, Park YS, Cho SY. 1993. Protective effect of *Oenanthe javanica* extract on the carbon tetrachloride-induced hepatotoxicity in mice. *J Korean Soc Food Nutr* 22: 392-397.
34. Bae SJ, Kim NH, Koh JB, Roh SB, Jung BM. 1997. Effects of Godulbaegi (*Ixeris Sonchifolia* H.) diet on enzyme activities of CCL_4 induced hepatotoxicity in rats. *Korean J Nutr* 30: 19-24.
35. Oh SH, Soh JR, Cha YS. 2003. Germinated brown rice extract shows a nutraceutical effect in the recovery of chronic alcohol-related symptoms. *J Med Food* 6: 115-121.
36. Cha YS, Oh SH. 2000. Investigation of γ -aminobutyric acid in Chinese cabbages and effects of the cabbage diets on lipid metabolism and liver funtion of rats administered with ethanol. *J Korean Soc Food Sci Nutr* 29: 500-505.
37. McGarry JD, Foster DW. 1980. Resolution of hepatic fatty acid oxidation and ketone body production. *Annu Rev Biochem* 49: 395-420.
38. McGarry JD, Brown NF. 1997. The mitochondrial carnitine palmitoyltranferase system: from concept to molecular analysis. *Eur J Biochem* 244: 1-14.
39. Bonnefont JP, Demaugre F, Prip-Buus C, Saudubray JM, Brivet M, Abadi, Thuiller L. 1999. Carnitine palmitoyltransferase deficiencies. *Mol Genet Matab* 68: 424-440.
40. Shon HS, Oh SH, Cha YS. 1999. Effects of exercise and/or high fat diet on carnitine and carnitine palmitoyltranferase-I mRNA levels in rats. *J Korean Soc Food Sci Nutr* 28: 670-676.

(Received April 7, 2003; Accepted June 5, 2003)